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Immunohistochemical subtypes predict the clinical outcome in high-risk node-negative breast cancer patients treated with adjuvant FEC regimen: results of a single-center retrospective study

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Abstract

Background: Anthracycline-based adjuvant chemotherapy improves survival in patients with high-risk node-negative breast cancer (BC). In this setting, prognostic factors predicting for treatment failure might help selecting among the different available cytotoxic combinations.

Methods: Between 1998 and 2008, 757 consecutive patients with node-negative BC treated in our institution with adjuvant FEC (5FU, epirubicin, cyclophosphamide) chemotherapy were identified. Data collection included demographic, clinico-pathological characteristics and treatment information. Molecular subtypes were derived from estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) status and Scarff-Bloom-Richardson (SBR) grade. Disease-free survival (DFS), distant disease-free survival (DDFS) and overall survival (OS) were estimated using the Kaplan-Meier Method, and prognostic factors were examined by multivariate Cox analysis.

Results: After a median follow-up of 70 months, the 5-year DFS, DDFS and OS were 90.6 % (95 % confidence interval (CI): 88.2–93.1), 92.8 % (95 % CI: 90.7–95) and 95.1 % (95 % CI, 93.3–96.9), respectively. In the multivariate analysis including classical clinico-pathological parameters, only grade 3 maintained a significant and independent adverse prognostic impact. In an alternative multivariate model where ER, PR and grade were replaced by molecular subtypes, only luminal B/HER2-negative and triple-negative subtypes were associated with reduced DFS and DDFS.

Conclusions: Node-negative BC patients receiving adjuvant FEC regimen have a favorable outcome. Luminal B/HER2-negative and triple-negative subtypes identify patients with a higher risk of treatment failure, which might warrant more aggressive systemic treatment.

Keywords: Breast cancer, Adjuvant chemotherapy, Anthracycline, Taxane, Peritumoral vascular invasion, Molecular subtypes, Prognostic factors, Node-negative

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Background

With more than 1.3 million of new cases annually and nearly 465,000 deaths, breast cancer (BC) is still the leading cause of cancer mortality among females in the world. In France, its incidence has been rising during the last 25 years and 53,000 new cases of BC were diagnosed in 2011. Adjuvant chemotherapy improves survival in early BC and is considered as one of the most significant therapeutic achievements in this disease during the last decades [1]. Initially, polychemotherapy, notably cyclophosphamide-methotrexate-5FU (CMF) regimen, was shown to significantly decrease relapses and deaths over monochemotherapy or no post-operative treatment at all [2, 3]. A few years later, anthracycline-based regimens were shown to further improve outcome over CMF, these effects being regarded as largely independent of main tumor characteristics such as estrogen receptor (ER) status, lymph node status and associated endocrine therapy [1]. Therefore, at the end of 90's, cyclophosphamide-epirubicin-5FU (FEC) regimen became the reference combination in France for adjuvant chemotherapy in early BC, epirubicin 100 mg/m² being shown as more effective than 50 mg/m² in a comparative randomized trial [4]. More recently, taxanes were incorporated to adjuvant anthracycline-based chemotherapy, with demonstrated benefits in disease-free survival (DFS) and overall survival (OS) when compared to anthracycline-based combinations in various comparative randomized trials [5, 6] and meta-analysis [7].

Most of the above described results were obtained from clinical trials enrolling essentially node-positive BC patients, but several studies also demonstrated a benefit for adjuvant chemotherapy in patients with high-risk, node-negative BC [8, 9], which was clearly confirmed by meta-analyses [3, 10]. When focusing specifically on anthracycline/taxane-based combinations, most of randomized trials enrolled only node-positive BC, while only few of them also included a limited number of node-negative BC. In fact, only two randomized phase III trials evaluating concomitant or sequential anthracycline/taxane-based combinations in a pure population of node-negative BC were recently reported [11, 12], both showing a benefit in DFS, but no clear superiority in OS. Thus, regarding to the significant increase in costs and acute toxicities, the use of these anthracycline/taxane-based regimens in node-negative BC remains controversial.

Here, we have conducted a retrospective analysis of a large single-center cohort of patients with high-risk node-negative BC treated with adjuvant FEC chemotherapy. Our primary objective was to identify prognostic factors for DFS, which might help better defining node-negative BC patients candidate to the most aggressive and costly anthracycline/taxane combinations. Specifically, we have examined the prognostic impact of

clinico-pathological parameters classically used to indicate adjuvant chemotherapy and that of immunohistochemically (IHC)-defined molecular subtypes of BC. Our secondary objectives were to describe OS and distant-disease free survival (DDFS) in this population. An additional secondary objective was to derive prognostic factors for DDFS.

Methods

Patient population

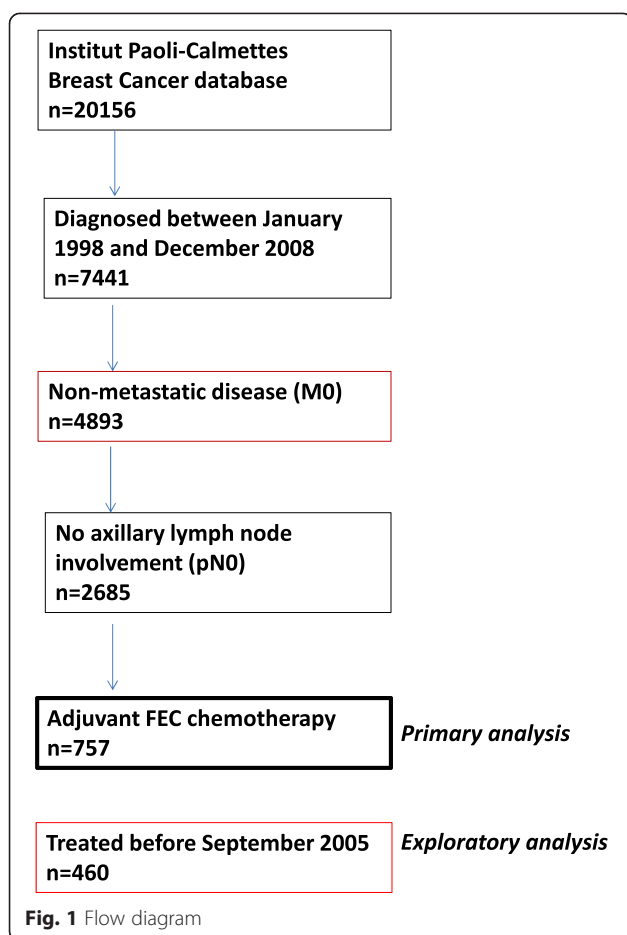
The study population was identified from our prospectively maintained institutional database. This institutional clinical data base on breast cancer is operated by DATA MANAGEMENT AND ANALYSIS CENTER (DMAC), approved for its data management skills as data processing centers by INCa (French National Institute for Cancer) in 2007. DMAC is also ISO 9001 certified. Standard operating procedures are applied to collect, check and use data in the quality management system framework. Data are entered (source is medical records) in an ORACLE database by Clinical Research Assistant.

The following inclusion criteria were used : all consecutive women diagnosed with invasive BC over a 11-year period between January 1998 and December 2008, treated with primary surgery (tumor resection and axillary staging by sentinel lymph node biopsy (SLNB) and/or axillary lymph node dissection (ALND)), pathologically node-negative (pN0) and treated with adjuvant FEC chemotherapy (at least 4 cycles of FEC50,75 or 100). Exclusion criteria were metastatic disease, node-positive or nodal status unknown or patient receiving neo-adjuvant treatment. A flow diagram of the population selection steps is provided in Fig. 1.

The study was approved by the Institut Paoli-Calmettes (IPC) Institutional Review Board (IRB, Comité d'Orientation Stratégique, COS). According to national guidelines, the IRB considered that there was no need for collecting informed consent for this kind of database study. Data were analyzed after all patient information have been fully anonymized.

Clinical and biological variables

The following patient's baseline characteristics were collected: age, type of surgery and axillary lymph node staging, pathological tumor size, histologic subtype, Scarf-Bloom-Richardson (SBR) grade (tubule formation, nuclear pleomorphism and mitotic count), peritumoral vascular invasion (PVI), IHC status for standard prognostic biomarkers : estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). For ER and PR, cases with 10 % or more positive staining were considered as positive. Hormone receptors (HoR) were considered as positive when ER and/or PR were positive. For HER2, the cases with 3+ staining by



IHC and/or amplification by in situ hybridization method were considered as positive.

Five molecular subtypes were defined according to clinico-pathologic criteria. Because information on Ki-67 was only partially available, we used SBR grade to capture cell proliferation, as previously described [13]. The following definitions were used: triple-negative (HoR-negative/HER2-negative/all grades), HER2-positive (HoR-negative/HER2-positive/all grades), luminal (HoR-positive), which was divided in luminal A (HoR-positive/HER2-negative/SBR grade 1 or 2), luminal B/HER2-negative (HoR-positive/HER2-negative/SBR grade 3), luminal B/HER2-positive (HoR-positive/HER2-positive, all grades).

Treatment procedures

Adjuvant chemotherapy was indicated for pN0 patients in case of high-risk features, defined as: pathological tumor size ≥ 15 mm, HoR-negative tumors, SBR grade 2 or 3, PVI, or age < 40 . FEC regimen was started within 12 weeks of surgery and included fluorouracil 500 mg/m², epirubicin 50-75-100 mg/m², cyclophosphamide 500 mg/m², administered every 21 days for 4–6 cycles.

Following completion of chemotherapy, patients were given adjuvant radiotherapy when appropriate and hormonal therapy in case of HoR-positivity (tamoxifen or aromatase inhibitors). From 2005, patients with HER2-positive BC were offered adjuvant trastuzumab after completion of radiotherapy.

Statistical analyses

The primary objective of this study was to identify prognostic factors for disease-free survival (DFS). Secondary objectives included description of distant DFS (DDFS) and overall survival (OS), as well as an exploratory analysis of prognostic factors for DDFS. Due to the low number of death events, no prognostic analysis was conducted for OS. DFS was defined as the time from diagnosis to local, node, contralateral or distant relapse or death from any cause. DDFS was defined as the time from diagnosis to distant relapse or death from any cause. OS was defined as the time from diagnosis to death from any cause. For patients who remained alive and disease-free, data were censored at the date of the last follow up.

Descriptive statistics were used to describe the categorical (counts and frequency) and continuous (median and ranges) variables. DFS, DDFS and OS rates were estimated using the Kaplan–Meier method and compared with the log-rank test. Multivariate analysis was conducted using Cox's proportional hazard regression model. All statistical tests were two-sided. The level of statistical significance was set at a P-value of 0.05. Statistical analyses were carried out with the R software version 2.15.2. We followed the “Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)” guidelines [14].

Results

Patient's characteristics

A total of 757 patients were identified (Fig. 1), the characteristics of which are presented in Table 1. Median age was 53 years (range, 24–79), more than half of patients had pT1 tumor (52 %), and most of tumors were HoR-positive (72 %), ductal histologic type (79 %), and 1 or 2 SBR grade (62 %). HER2 status was available for 80 % of cases and was positive in 16 % of those cases. Most of patients had breast-conserving surgery (73 %), received adjuvant radiation therapy (90 %) and hormonal therapy (70 %). Almost all patients received FEC100 regimen (94 %), FEC75 or FEC50 being usually administered if age was more than 70. Of note, 41 % of patients with HER2-positive documented BC received trastuzumab after chemotherapy and radiotherapy completion, corresponding to 85 % of HER2-positive cases diagnosed after September 2005, when trastuzumab became available in the adjuvant setting in France, but less than 5 % of those diagnosed before this date. Reasons for not receiving

Table 1 Patient characteristics

Age (Years)	Median [range]	53 (24–79)	
	<35 (%)	30 (4 %)	
	≥70 (%)	43 (6 %)	
Type of surgery	Lumpectomy	552 (73 %)	
	Complete mastectomy	205 (27 %)	
Axillary management	Lymph node dissection	395 (52 %)	
	Sentinel lymph node biopsy	362 (48 %)	
Histological type	Invasive ductal carcinoma	599 (79 %)	
	Invasive lobular carcinoma	66 (9 %)	
	Other invasive carcinoma	92 (12 %)	
Pathological tumor size (pT)	pT1a-b	63 (8 %)	
	pT1c	329 (44 %)	
	pT2	320 (43 %)	
	pT3-T4	36 (5 %)	
SBR grade	1	132 (18 %)	
	2	331 (44 %)	
	3	285 (38 %)	
	NA	9	
Peritumoral vascular invasion (PVI)	No	558 (76 %)	
	Yes	174 (24 %)	
	NA	25	
Hormone receptors			
	ER	Negative	229 (31 %)
		Positive	506 (69 %)
NA		32	
PR	Negative	287 (39 %)	
	positive	441 (61 %)	
	NA	29	
ER or PR	Negative	208 (28 %)	
	Positive	527 (72 %)	
	NA	22	
HER2 status	Positive	96 (16 %)	
	Negative	508 (84 %)	
	NA	153	
IHC subtypes	Triple-negative	127 (21 %)	
	Luminal A	290 (49 %)	
	Luminal B/HER2-negative	82 (14 %)	
	Luminal B/HER2-positive	58 (10 %)	
	HER2	37 (6 %)	
	NA	163	
Adjuvant chemotherapy	FEC 100	716 (94 %)	
	FEC 50/75	41 (6 %)	
Adjuvant hormone therapy	Yes	532 (70 %)	
	No	224 (30 %)	
	NA	1	

Table 1 Patient characteristics (*Continued*)

Adjuvant radiation therapy	Yes	681 (90 %)
	No	76 (10 %)
Adjuvant trastuzumab (HER2-positive)	Yes	40 (41 %)
	No	56 (59 %)

trastuzumab after September 2005 were: heart failure ($n = 1$), patient refusal ($n = 1$), discordant or equivocal pathological result ($n = 2$), unknown ($n = 3$).

Outcome

The estimated median follow-up was 70 months (95 % confidence interval (CI): 63.5–75.0). Sixty-nine DFS events, including 38 distant metastases and 39 deaths were observed. The estimated 5-year DFS, primary endpoint of this study, was 90.6 % (95 % CI: 88.2–93.1; Fig. 2A). Regarding the secondary outcome endpoints, the estimated 5-year DDFS was 92.8 % (95 % CI: 90.7–95; Fig. 2B) and 5-year OS was 95.1 % (95 % CI: 93.3–96.9; Fig. 2C).

Clinico-pathological predictors of DFS

In the univariate analysis, the following parameters were associated with a reduced DFS (Table 2): SBR grade 3 (hazard ratio (HR) = 1.8, 95 % CI [1.1–2.9], $p = 0.0138$), PVI (HR = 2.3, 95 % CI [1.4–3.7], $p = 0.0007$), and IHC subtypes ($p = 0.0006$). As for the latter, luminal B/HER2-negative (HR = 4.3, 95 % CI [2–9.1]) and triple-negative (HR = 2.6, 95 % CI [1.2–5.5]) subtypes were associated with an adverse outcome (Fig. 3). Of note, pathological tumor size, HER2 and ER status were not prognostic in this treated population. In the multivariate analysis, only grade 3 (HR = 2.8, 95 % CI [1.4–5.4], $p = 0.0027$) retained an independent prognostic value (Table 3). In an alternative multivariate Cox model in which ER, PR and grade were replaced by IHC subtypes, only luminal B/HER2-negative (HR = 4.25, 95 % CI [1.9–9.15], $p = 0.0002$) and triple-negative (HR = 2.5, 95 % CI [1.15–5.4], $p = 0.0201$) subtypes were independently associated with an adverse outcome (Table 3).

Since a large part of patients with documented HER2-positive BC received adjuvant trastuzumab after September 2005, we performed an exploratory analysis focused on patients treated before this period ($n = 460$). In this subset of patients, HER2 was still not significantly associated with DFS, and the same variables (grade 3, PVI, IHC subtypes) were found to be predictive in the univariate analysis (See Additional file 1). In the multivariate analysis, SBR grade 3 remained significantly associated with poor DFS, while a similar trend was observed for PVI (See Additional file 2). Once again, IHC subtypes (Luminal B/HER2-negative and, with borderline significance, triple-negative), instead of

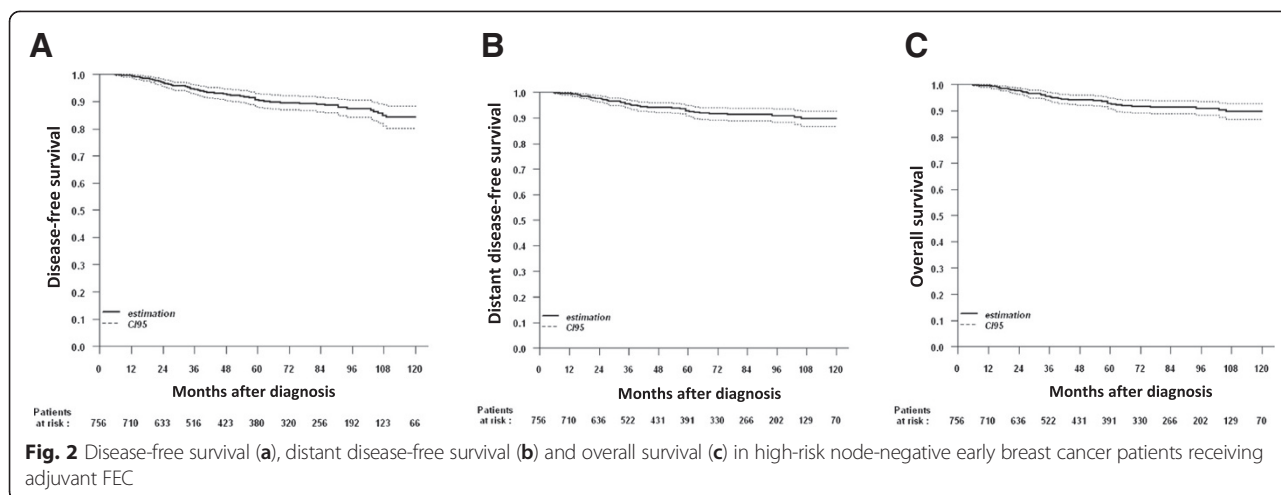
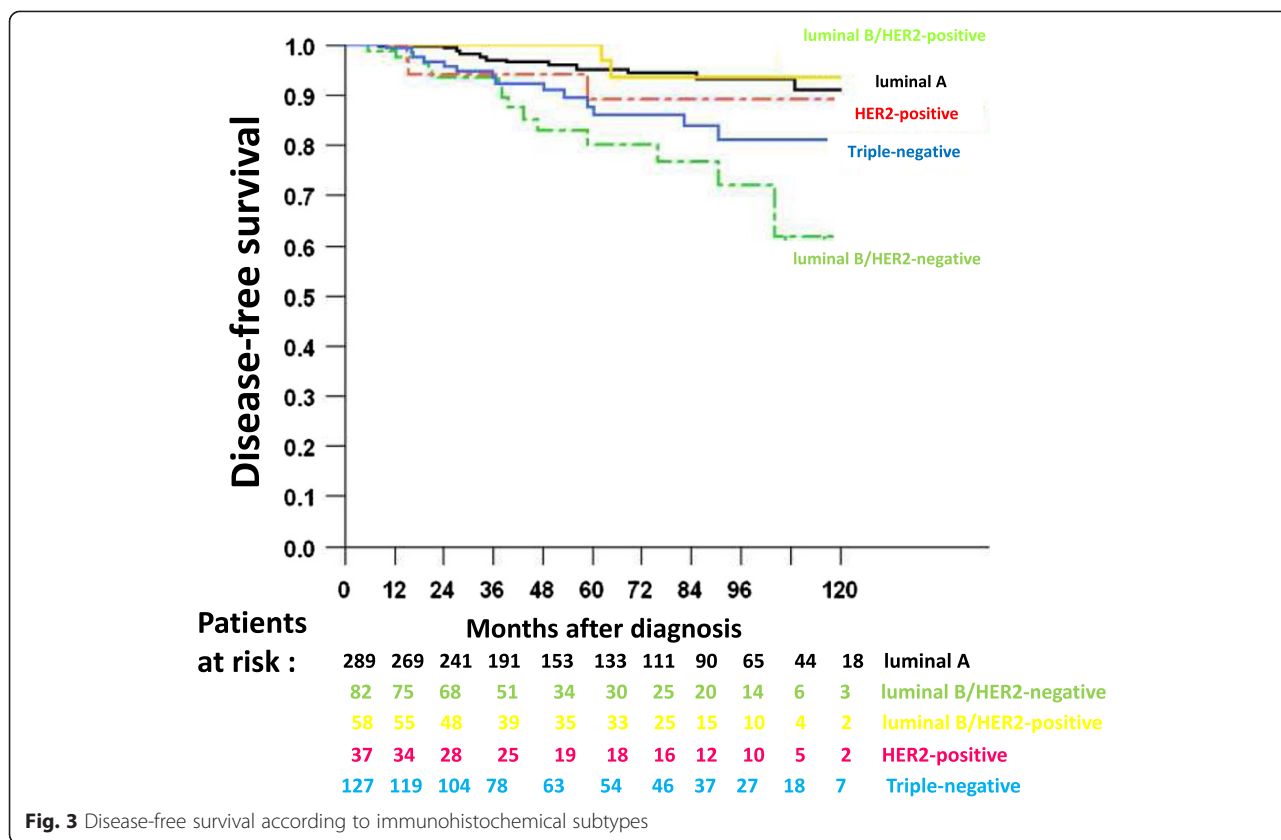


Table 2 Prognostic factors for disease-free survival (DFS): univariate analysis

	N	5-Year DFS (%) (95 % CI)	HR (95 % CI)	p-value ^a
Age				
< 35	30	82.6 [68.2–100]	1	
≥ 35	726	90.9 [88.5–93.4]	0.5 [0.2–1.3]	0.136
SBR Grade				
1–2	462	93.2 [90.5–95.9]	1	
3	285	86 [81.4–91]	1.8 [1.1–2.9]	0.0138
Pathological tumor size				
pT1	391	91.2 [87.9–94.5]	1	
pT2	320	90 [86.2–94]	1.1 [0.7–1.8]	
pT3-T4	36	88.2 [78–99.8]	1.1 [0.4–3]	0.9514
PVI				
No	558	92.3 [89.7–95]	1	
Yes	178	85 [79–91.4]	2.3 [1.4–3.7]	0.0007
Hormone receptors				
Negative	208	86.8 [81.6–92.4]	1	
Positive	526	92.5 [89.9–95.2]	0.7 [0.4–1.1]	0.1059
HER2				
Negative	507	91 [88–94.1]	1	
Positive	96	95.7 [91–100]	0.6 [0.2–1.6]	0.376
IHC subtypes				
Luminal A	289	95.1 [92.1–98.2]	1	
Luminal B/HER2-negative	82	80.1 [69.8–92]	4.3 [2–9.1]	
Luminal B/HER2-positive	58	100 [100–100]	0.7 [0.2–3.3]	
HER2	37	89 [77.6–100]	1.8 [0.5–6.2]	
Triple-negative	127	87.6 [80.7–95]	2.6 [1.2–5.5]	0.0006

HR hazard ratio, PVI peritumor vascular invasion

^aunadjusted log-rank test



SBR grade, HER2 and HR, were independently associated with DFS (Additional file 2: Table S2).

Clinico-pathological predictors of DDFS

When looking at variables associated with DDFS, SBR grade 3 (HR = 2.8, 95 % CI [1.6–4.9], p = 0.0002), PVI (HR = 2.2, 95 % CI [1.3–4], p = 0.0042), lack of hormone receptor expression (HR = 0.6, 95 % CI [0.3–1], p = 0.049), and luminal B/HER2-negative (HR = 4.6, 95 % CI [1.8–11.7]) and triple-negative (HR = 3.3, 95 % CI [1.3–8.2]) IHC subtypes (p = 0.0059) were associated with reduced DDFS in the univariate analysis (Table 4). In the multivariate analysis, only grade (HR = 3.4 95 % CI [1.5–7.6], p = 0.0027) had an independent value (Table 5). Again, when replacing hormone receptor and grade by IHC subtypes, only the latter had an independent prognostic value, with luminal B/HER2-negative and triple-negative subtypes being associated with an adverse outcome (Table 5).

Discussion

In this paper, we have examined the clinical outcome of a large, single-centre, retrospective cohort of patients with high-risk node-negative early BC receiving post-operative FEC-based chemotherapy. With a 5-year DFS of more than 90 %, and a 5-year OS of more than 95 %,

overall prognosis was considered to be excellent, but some subsets of patients were identified as displaying a less favorable outcome. Namely, the presence of a SBR grade 3 still predicted for a higher risk of relapse. A similar independent dismal prognosis was identified for luminal B/HER2-negative and triple-negative subtypes, suggesting that these patients might be the best candidate to more aggressive adjuvant chemotherapy, including the more aggressive and costly anthracycline and taxane-based combination.

Histologic grade is a semi-quantitative estimation of the degree of tubular or gland formation, nuclear polymorphism and mitotic count [15], the independent prognostic impact of which was recurrently demonstrated in various settings [16], including node-negative or node-positive disease [17]. Accordingly, high grade has been considered as a major determinant for adjuvant chemotherapy decision, which is related at least partially to its dependency on cell proliferation and mitosis. Here, we have shown that SBR grade still retains an independent adverse prognostic value, in the context of node-negative BC patients receiving anthracycline-based adjuvant chemotherapy. Thus, even though it does not seem to predict a specific sensitivity to a particular class of drug, it may indicate a subset of BC patients in which adding taxanes

Table 3 Prognostic factors for disease-free survival: multivariate analysis

	Model 1 (N = 570)		Model 2 (N = 575)	
	HR (95 % CI)	<i>p</i> -value ^a	HR (95 % CI)	<i>p</i> -value ^a
Age				
< 35	1		1	
≥ 35	0.5 [0.1–2.3]	0.581	0.5 [0.1–.396]	0.440
SBR Grade				
1–2	1		NA	
3	2.8 [1.4–5.4]	0.0027	NA	
Pathological tumor size				
pT1	1		1	
pT2	0.9 [0.5–1.7]	0.897	0.9 [0.5–1.7]	0.916
pT3-T4	0.4 [0.05–3.3]	0.431	0.4 [0.06–3.4]	0.447
PVI				
No	1		1	
Yes	1.5 [0.8–3.0]	0.175	1.3 [0.7–2.6]	0.357
Hormone receptors				
Negative	1		NA	
Positive	1.0 [0.5–2.2]	0.818	NA	
HER2				
Positive	1		NA	
Negative	0.5 [0.2–1.4]	0.239	NA	
IHC subtypes				
Luminal A	NA		1	
Luminal B/HER2-negative	NA		4.2 [1.9–9.1]	0.0002
Luminal B/HER2-positive	NA		0.7 [0.1–3.4]	0.721
HER2	NA		1.76 [0.4–6.1]	0.38
Triple-negative	NA		2.5 [1.1–5.4]	0.0201

HR hazard ratio, PVI peritumor vascular invasion

^aWald test

to anthracyclines may be of interest in spite of the absence of lymph node invasion.

PVI was shown to be an adverse prognostic parameter in early BC, notably in node-negative BC, where it has been considered as a surrogate for occult vascular dissemination [18]. Its prognostic value has been also described within the different breast cancer subtypes [19, 20]. In our study, whereas it was consistently associated with DFS and DDFS, it was not retained as an independent prognosticator in all multivariate models tested. That suggests that, in the context anthracycline-based adjuvant CT, the unfavorable prognostic impact of PVI is not as important as proliferation status and subtypes. This lack of prognostic value in multivariate analysis including breast cancer subtypes could also be explained by the fact that PVI was recently shown to correlate with the luminal B/HER2-negative subtype [21].

Of note, age and pathological tumor size were not associated with survival, although being classical

prognosticators currently used to recommend adjuvant chemotherapy. Thus, following anthracycline-based adjuvant chemotherapy, prognosis and therefore the potential need for taxane-based treatment, may more result from biological rather than from pure clinical features.

Notably, the lack of hormone receptors and HER2 overexpression were not individually associated with a significantly higher risk of relapse. This may be related to the classically documented efficacy of anthracycline-based adjuvant chemotherapy in HR-negative tumors [1, 3]. An explanation for the relative favorable outcome of patients with HER2 overexpression may be that a significant fraction of those patients received, after completion of chemotherapy and radiotherapy, adjuvant trastuzumab, the impact of which was largely demonstrated during the last 10 years [22, 23]. However, a sensitivity analysis focused on patients treated before 2005 September (date of initiation of adjuvant trastuzumab in

Table 4 Prognostic factors for distant disease-free survival: univariate analysis

	N	5-Year DDFS (%) (95 % CI)	HR (95 % CI)	<i>p</i> -value ^a
Age				
< 35	30	82.6 [68.2–100]	1	
≥ 35	726	93.3 [91.2–95.4]	0.5 [0.2–1.3]	0.1223
SBR Grade				
1–2	462	96 [94–98]	1	
3	285	87.4 [82.9–92.1]	2.8 [1.6–4.9]	0.0002
Pathological tumor size				
pT1	391	93.6 [90.9–96.5]	1	
pT2	320	92.3 [88.9–95.7]	1.2 [0.7–2]	
pT3-T4	36	88.2 [78–99.8]	1.6 [0.6–4.7]	0.6395
PVI				
No	558	94.2 [92–96.5]	1	
Yes	178	88.8 [83.5–94.5]	2.2 [1.3–4]	0.0042
Hormone receptors				
Negative	208	88 [83–93.4]	1	
Positive	526	94.7 [92.5–96.9]	0.6 [0.3–1]	0.049
HER2				
Negative	507	93.2 [90.5–95.9]	1	
Positive	96	95.7 [91–100]	0.9 [0.3–2.2]	0.7719
IHC subtypes				
Luminal A	289	96.8 [94.5–99.2]	1	
Luminal B/HER2-negative	82	85.5 [76.3–95.8]	4.6 [1.8–11.7]	
Luminal B/HER2-positive	58	100 [100–100]	1.2 [0.2–5.5]	
HER2	37	89 [77.6–100]	2.9 [0.8–11.1]	
Triple-negative	127	89 [82.5–96]	3.3 [1.3–8.2]	0.0059

HR hazard ratio, PVI peritumor vascular invasion

^aunadjusted log-rank test

HER2-positive BC at our institution) did not show a significant adverse impact of HER2-overexpression in this population. An alternative hypothesis could be the suspected link between *HER2* amplification and sensitivity to anthracycline-based regimen [24, 25], possibly due to frequent co-amplification or deletion of *TOP2A*, the gene encoding topoisomerase II α protein, which is a supposed intracellular target for anthracyclines [26].

Results from transcriptomic studies have shown that BC is an heterogeneous disease that can be divided in at least 5 molecular subtypes (basal-like, HER2-enriched, luminal A, luminal B and normal-like) with specific gene expression patterns, distinct clinical outcome and sensitivity to systemic treatments [27]. Accordingly, it has been hypothesized that identifying these BC subtypes in the clinic could help better tailoring individual therapeutic choices. Since molecular subtypes are only precisely identified by sophisticated gene expression profiling technologies not readily applicable to clinical

samples in routine, it has become increasingly popular to approximate these subtypes using IHC evaluation of ER, PR and HER2 expression, combined to a surrogate of the proliferation status such as Ki-67 expression or grade. The main objective of this subtype-based classification has been to identify patients with low or no tumor burden in the axillary lymph node and a relatively indolent disease, who could be spared from adjuvant cytotoxic treatment [28]. Thus, according to this classification, most of luminal A tumors need endocrine but not cytotoxic treatment, whereas adjuvant chemotherapy is recommended in tumors identified as triple-negative, luminal B (followed by endocrine treatment), and HER2 (with trastuzumab). In this study, we have used IHC subtypes to identify patients with node-negative BC receiving adjuvant FEC who may have still a significant residual risk of relapse. Triple-negative and luminal-B/HER2-negative subtypes displayed the worst outcome, including a distant relapse risk which was higher than 10 % in both cases,

Table 5 Prognostic factors for distant disease-free survival: multivariate analysis

	Model 1 (N = 570)		Model 2 (N = 575)	
	HR (95 % CI)	<i>p</i> -value ^a	HR (95 % CI)	<i>p</i> -value ^a
Age				
< 35	1		1	
≥ 35	0.4 [0.1–2.1]	0.344	0.4 [0.09–1.8]	0.251
SBR Grade				
1–2	1		NA	
3	3.4 [1.5–7.6]	0.0027	NA	
Pathological tumor size				
pT1	1		1	
pT2	1.0 [0.5–2.0]	0.932	1.0 [0.5–2.1]	0.847
pT3-T4	0.7 [0.09–5.3]	0.733	0.7 [0.09–5.5]	0.763
PVI				
No	1		1	
Yes	1.7 [0.8–3.6]	0.155	1.5 [0.7–3.2]	0.275
Hormone receptors				
Negative	1		NA	
Positive	0.9 [0.4–2.0]	0.865	NA	
HER2				
Positive	1		NA	
Negative	0.7 [0.2–2.0]	0.605	NA	
IHC subtypes				
Luminal A	NA		1	
Luminal B-HER2-negative	NA		4.4 [1.7–11.5]	0.0018
Luminal B-HER2-positive	NA		1.2 [0.2–5.9]	0.775
HER2	NA		2.8 [0.7–10.9]	0.118
Triple-negative	NA		3.4 [1.3–8.7]	0.0074

HR hazard ratio, PVI peritumor vascular invasion

^aWald test

theoretically justifying a more aggressive systemic treatment. Triple-negative status is a very well-known poor prognostic factor in early BC and some retrospective analyses of randomized studies have shown that this subset of tumors may derive a pronounced benefit from taxane addition, including node-negative BC [29–31]. However, the triple-negative subtype has been shown to be heterogeneous at the molecular level, including various subtypes with distinct clinical behavior and phenotype of drug sensitivity [32]. Specifically, recent data have identified a favorable prognosis in triple-negative BC receiving anthracycline-based adjuvant chemotherapy and displaying a high level of lymphocytic infiltration [33, 34].

Notably and surprisingly, the luminal B/HER2-negative subtype was found to be even more aggressive, with a 5-year risk of relapse of 20 %, including a risk of distant relapse of nearly 15 %. The aggressive clinical

behavior of Luminal B tumors is well known and their prognosis has been considered as similar to that of HER2-enriched and basal-like groups [35]. However, while triple-negative and Luminal B/HER2-positive tumors might derive higher benefits from anthracyclines and/or anti-HER2 adjuvant treatment, some Luminal B/HER2-negative could be sub-optimally treated with anthracycline-only regimen. Thus, these results suggest that these tumors should also be candidate to more aggressive chemotherapy with addition of taxanes to anthracycline-based combination. Of note, in the French PACS 01 study, which compared in node-positive BC treated in the adjuvant setting 6 cycles of FEC100 versus 3 cycles FEC100 followed by 3 cycles of docetaxel, luminal B tumors were shown to derive the highest benefit from taxane addition [36].

Even though they were not found to display a significantly higher risk of relapse in our study (which could be

due to the relatively limited sample size of this subgroup), HER2-positive subtypes are also pragmatic candidates to taxane, since it is a way to earlier initiate trastuzumab, either after a limited anthracycline-based sequence or at the beginning of chemotherapy in the context of anthracycline-free combinations. Indeed, early initiation of trastuzumab has been associated with better outcome, while reducing anthracycline exposure may limit cardiac toxicity [37]. Finally, with a risk of distant relapse of less than 4 %, luminal A BC had an excellent outcome and the most relevant question in this subset of patients, sensitive to hormone therapy, is whether these patients do actually need any cytotoxic treatment. Various multigenic signatures are now available that are specifically addressing this issue [38, 39] and the prospective validation of some of them is currently under analysis [40, 41].

This study has several strengths lying in the number of samples analyzed (more than 750), the duration of the follow-up (nearly 8 years in median) and the high-quality of data collected by a certified Data Management and Analysis Center. Weaknesses include its retrospective monocentric, non-randomized and non-comparative nature, the lack of central and ad hoc review of biological variables, the relatively high number of missing data for analysis of molecular subtypes ($n = 163$), notably the number of missing HER2 status, the discussable approximation of luminal A/B distinction based on grade only, rather than on Ki67 and the low number of death events, precluding the identification of prognostic factors for OS. Another potential bias may be the definition of high-risk features used to indicate adjuvant chemotherapy during this period (pathological tumor size ≥ 15 mm, HoR-negative tumors, SBR grade 2 or 3, PVI, or age < 40), which is not the same as currently used. Finally, the relatively low number of HER2-positive patients receiving trastuzumab (49 %) may also affect the clinical relevance of our results in routine practice.

In spite of the above-mentioned limitations, our main result suggests that triple-negative and luminal-B/HER2-negative subtypes have a significant residual risk of relapse following FEC adjuvant chemotherapy. This may support the use of taxanes in these subsets of patients, even when node-negative, but additional researches are warranted in order to better predict a specific therapeutic benefit of this class of drugs according to the tumor features.

Conclusions

In conclusion, our results suggest a relative favorable outcome in node-negative BC treated with FEC-based adjuvant chemotherapy. However, SBR grade 3 or triple-negative and luminal-B/HER2-negative subtypes may indicate which patients are candidates to anthracycline and taxane-based combinations.

Additional files

Additional file 1: Prognostic factors for disease-free survival in patients treated before 2005 September: univariate analysis. (DOCX 15 kb)

Additional file 2: Prognostic factors for disease-free survival in patients treated before 2005 September: multivariate analysis. (DOCX 15 kb)

Abbreviations

ALND: Axillary lymph node dissection; BC: Breast cancer; CI: Confidence interval; CMF: Cyclophosphamide, methotrexate, 5FU; COS: Comité d'Orientation Stratégique; DDFS: Distant disease-free survival; DFS: Disease-free survival; DMAC: Data management and analysis center; ER: Estrogen receptor; FEC: 5FU, Epirubicin, Cyclophosphamide; HER2: Human epidermal growth receptor 2; HoR: Hormone receptors; IHC: Immunohistochemistry; INCa: French National Institute for Cancer; IPC: Institut Paoli-Calmettes; IRB: Institutional Review Board; OS: Overall survival; pNO: Pathologically node-negative; PR: Progesterone receptor; PVI: Peritumoral vascular invasion; SBR: Scarff-Bloom-Richardson; SLNB: Sentinel lymph node biopsy; STROBE: Strengthening the Reporting of Observational Studies in Epidemiology; TOP2A: Topoisomerase IIa.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AG conceived and coordinated the study, participated to data collection and analysis and wrote the manuscript. SR, JMB, FB, RS, ECJ, LL and MR participated to data collection and analysis and helped to draft manuscript. JME, CT, EL, JTP, AT, GH and PV participated to data collection and analysis. All authors read and approved the final manuscript.

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